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BRIEF REPORT

Systematic screening for mutations in the glycine receptor $\alpha 2$ subunit gene (GLRA2) in patients with schizophrenia and other psychiatric diseases

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The glycine receptor, which is a member of the ligand-gated ion channel superfamily, mediates synaptic inhibition in the spinal cord and other brain regions. This superfamily has been implicated in the pathogenesis of schizophrenia and other psychiatric diseases. The complete coding sequence and splice junctions of the GLRA2 gene were scanned by DOVAM-S, a form of SSCP analysis with sufficient redundancy to detect virtually all mutations. Those analyses were performed in 113 patients with schizophrenia, and in pilot studies of patients with bipolar illness, alcoholism, puerperal psychosis, autism, and attention-deficit hyperactivity disorder (533 kb total scanned sequences). We detected three sequence changes in the coding region, all resulting in silent mutations: C894T in exon 5, C1134T in exon 7, and C1476T in exon 9. These do not alter the structure or the expression of the protein. It is unlikely that mutations in the coding region and splice junction of GLRA2 gene are associated with schizophrenia and other psychiatric diseases. Psychiatr Genet 11:45–48 © 2001 Lippincott Williams & Wilkins.

Keywords: GLRA2, schizophrenia, detection of virtually all mutations–SSCP

INTRODUCTION

Glycine is a major inhibitory neurotransmitter in the central nervous system. Its action is mediated by a specific glycine receptor, which is a member of the ligand-gated ion channel superfamily that includes receptors for acetylcholine, γ-aminobutyric acid, serotonin, and glutamate (Vannier and Triller, 1997). These receptors are composed of five subunits arranged to form a central ion channel in the postsynaptic membrane. Each subunit consists of a large N-terminal extracellular domain, in which ligand binding sites reside, and four transmembrane domains that form the receptor channel (Grenningloh *et al*., 1987). In the human, highly homologous $\alpha 1$ and $\alpha 2$ subunits (452 amino acids) of the glycine receptor have been identified by cDNA cloning (Grenningloh *et al*., 1990) and assigned to the chromosomal regions 5q31.3 and Xp21.2-22.1, respectively. The glycine receptor may play a role in the negative symptoms of schizophrenia (Semba, 1998). In this study, we test the hypothesis that mutations within the glycine receptor $\alpha 2$ subunit gene (GLRA2) are associated with schizophrenia or other psychiatric diseases.

MATERIALS AND METHODS

Patient samples

All schizophrenic patients met criteria for the disease as defined by the Diagnostic and Statistical Manual, Third Edition, Revised (DSM-III-R), and described previously (Sobell *et al*., 1993). The majority of patients were ascertained through state mental institutions in Minnesota, Washington, and Oregon. Three schizophrenic patients from the Costa

Ricans' population isolate (DeLisi *et al*., 2000) and three schizophrenic patients from a Finnish population isolate were also examined. Caucasian females who had experienced at least one episode of puerperal psychosis were recruited through the Division of Neuroscience at the University of Birmingham in the UK (Robertson *et al*., 2001). Patients with bipolar disorder were ascertained from the UK (Jones *et al*., 2000) and a population isolate in Finland. Patients with alcoholism of Finnish ethnicity were ascertained through collaborative efforts involving the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the University of Helsinki, Finland. Southwestern Native American patients with alcohol dependence were acquired through the NIAAA (Feng *et al*., 1998a), and patients with autistic disorder or attention deficit hyperactivity disorder (ADHD) were ascertained from the University of Chicago (Cook *et al*., 1995, 1998).

Polymerase chain reaction amplification and detection of virtually all mutations–SSCP

Genomic DNA and standard polymerase chain reaction (PCR) conditions (Buzin *et al*., 2000) were used to generate nine separate PCR segments labeled with [α^{33}P]dATP (Amersham, Boston, MA, USA) that included all coding sequences and splice junctions. The segments were amplified robotically on the ABI PRISM™ 877 integrated thermal cycler (Applied Biosystems, Inc., Foster City, CA, USA), and automatically pooled into a single tube. The PCR primers are presented in Table 1. The reference sequence was taken from Cummings *et al*. (1998) (Genbank accession number, AF053487–AF053495). Following PCR amplification, the products were electrophoresed through non-denaturing acrylamide gels using detection of virtually all mutations–SSCP (DOVAM-S). Segments with mobility shifts were re-amplified and sequenced in both directions by an ABI 377 Automated Sequencer (Applied Biosystems, Inc.). Sequence chromatograms were analyzed with Sequencher™ software (Gene Codes, Ann Arbor, MI, USA).

DOVAM-S, a form of multi-conditional SSCP utilizing a generic set of five conditions, has successfully detected all of 250 mutations and polymorphisms in three blinded analyses of the factor VIII, factor IX and ATM genes (Buzin *et al*., 2000; Liu *et al*., 1999). The conditions are: Page^{plus}/capso/4°C, Page^{plus}/Tri/Tri/20°C, Page^{plus}/TBE/5% glycerol/20°C, HR1000/TBE/2.5% glycerol/4°C, HR1000/Tri/Tri/4°C (Buzin *et al*., 2000).

The five conditions provide enough redundancy to detect virtually 100% of single base changes and small deletions and insertions. However, large heterozygous deletions, insertions and rearrangements will not be detected in this or most of the PCR-based

TABLE 1. PCR primers for *GLRA2* gene

Primer name*	Sequence (5′ to 3′)	Segment size (base pairs)
E1(464)-18D	TAT TTC CAC AAG CAA CAC	176
E1(639)-20U	AGC ACA GAT AAA TAC ACA AT	
E2(166)-22D	ATG AAA TTG TTG CTA AAA GAG T	212
E2(377)-20U	AGA TTT CTC AAA GGC TTA AC	
E3(197)-17D	AGC CAA TTG CAC AGA TG	344
E3(540)-18U	CAT TCC TTC ACA CGA TGG	
E4(224)-20D	GTA ATC AGT AAC ACT TGT CC	300
E4(523)-18U	AGA AAT GGA GAT GCG AAC	
E5(249)-20D	GGC AAG TGT ATT TTG TTT GT	192
E5(440)-19U	GAG TCA TTT TCA ACA GTG G	
E6(288)-20D	AAG CCA TAT TCA ATT TTC AC	228
E6(515)-18U	ATG GCG CTC ACT TGT GTT	
E7(260)-20D	ATA TAA ATT CAG GCT GGA CT	289
E7(548)-20U	GTC ATC TGT TAT CAA GCA AG	
E8(135)-18D	GTT TTC CTG GCA GGC TTT	259
E8(393)-18U	CAT TGT CTG AAC TGA GGG	
E9(102)-19D	ATC TTC CAT CTA ACT TGA C	369
E9(470)-18U	GCA AGA AGG TCC CAG GGT	

*This nomenclature (Sarkar and Sommer, 1989) defines an oligonucleotide specific for the *GLRA2* gene: 'E' specifies the exon; the value in parentheses is the nucleotide number in the reference sequence; the number following the hyphen is the length of the primer in nucleotides; and the final letter indicates the orientation in the downstream direction (D) or the upstream direction (U).

methods, since only the normal allele will be amplified.

RESULTS AND DISCUSSION

The complete coding sequence and splice junctions of the GLRA2 gene were analyzed in 225 subjects comprised of 113 patients with schizophrenia, 28 with bipolar illness (BPI), 24 with puerperal psychosis, five with autism, 30 with ADHD, and 25 with alcoholism. A total of 533 kb GLRA2 genes were scanned. Three silent mutations in the coding region were found: C894T (Thr169Thr) in exon 5, C1134T (His249His) in exon 7, and C1476T (Asp363Asp) in exon 9. C1134T is a common polymorphism occurring at a high frequency as previously reported (Cummings *et al*., 1998). C894T was found in two patients with schizophrenia, one with alcoholism and one with BPI. C1476T was found in one schizophrenia patient only. The latter two sequence changes are novel, but low in frequency and not associated with the generation of cryptic splice consensus sequences.

No variants affecting protein structure or expression (VAPSEs) were observed in the dopamine D1 receptor and the thyroxin α receptor when 668 and 1168 kb, respectively, of sequences were scanned in patients with psychiatric diseases (Liu *et al*., 1995. Feng *et al*., 1998a, 2001). In contrast, four to six VAPSEs have been observed on scanning of 600–1200 kb of sequence for the α2 adrenergic receptor (Feng *et al*., 1998b), the dopamine D5 receptor (Sobell *et al*., 1995; Feng *et al*., 1998a), glucocorticoid receptor (Feng *et al*., 2000), and the estrogen receptor α (Feng *et al*., 2001).

The absence of any candidate mutations in 225 patients indicates that the GLRA2 gene mutations in coding regions and splice junctions are not associated with schizophrenia or other psychiatric diseases, at least in the populations and ethnic groups examined. Since the patient ascertainment criteria (psychiatric diseases) are not strongly related to mutations in the gene, the patients may be similar to a random sample of their ethnic groups. The absence of any candidate mutations in the GLRA2 gene in a large sample of patients suggests that mutations in this gene are not commonly associated with genetic predisposition to any of the common multi-factorial diseases.

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